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ORIGINAL ARTICLE

The isolation of novel terrestrial *Streptomyces* strains with antimicrobial and cytotoxic properties

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ABSTRACT

Streptomyces are very important Gram-positive filamentous bacteria because they can produce many beneficial secondary metabolites with various biological activities. The study aims to isolate and identify novel *Streptomyces* strains from terrestrial habitats in the United Arab Emirates (UAE) with antimicrobial and cytotoxic properties. The antimicrobial efficacy was assessed using the disk and well susceptibility methods. Crude ethyl acetate extracts of active-producing strains were used to determine the antimicrobial activity by minimum inhibitory concentration (MIC) against *E. coli*, *S. aureus, C. albicans*, *S. cerevisiae*, *B. subtilis,* and *E. coli*-ESBL (extended-spectrum Beta-Lactamase), and cytotoxic properties against HeLa cells by MTT assay and DAPI staining. The novelty of the strains was determined by sequencing the 16S rRNA fragment. Results showed that antimicrobial activity was observed for CSK1, CSK3, CSW2, CSU1, CSU2, and CSG1 strains, with zones of inhibition ranging between 16 and 35 mm. Minimum inhibitory concentrations of ethyl acetate extracts ranged from 0.21 to 12.17 µg/mL, with the highest inhibitory effect against *S. aureus* ranging between 0.21 and 0.29 µg/mL. Some strains (CSK3, CSW2, CSU1 and CSG1) also displayed cytotoxic activity against the HeLa cancer cell line with IC50 values ranging between 3.46 and 9.74 µg/µL, and apparent DNA fragmentation and chromatin condensation. This study indicates that *Streptomyces sp.* strains isolated from different soil habitats in the UAE can produce antimicrobial compounds that can treat microbial infections. In addition, some strains' cytotoxicity suggests that they are producing bioactive compounds that can lead to drug discovery.

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Antimicrobial metabolites; cytotoxic agents; *Streptomyces*; terrestrial habitats

1. Introduction

Gram-positive filamentous bacteria known as *Streptomyces* are capable of forming aerial mycelium and spores. They belong to the family *Streptomycetaceae* and are significant due to their ability to produce secondary metabolites that elicit specific bioactivities (Donald et al., 2022). *Streptomyces* produces approximately 40% of all bioactive secondary metabolites, making this genus extremely important in the field of drug discovery (Lacey & Rutledge, 2022). Over 6,000 bioactive secondary metabolites produced by various *Streptomyces* species have been used for their inhibitory properties in clinical applications that include antimicrobials (erythromycin, streptomycin; Alam et al., 2022), antifungals (amphotericin B; Carolus et al., 2020), antiviral (virantmycin; Kimura et al., 2019), or anticancer agents (doxorubicin and bleomycin; Zaid et al., 2021). The isolation, identification and characterization of new *Streptomyces* strains continue to be a burgeoning field of discovery; such is its importance to clinical medicine (Nguyen et al., 2020).

Although *Streptomyces* have been isolated from numerous terrestrial habitats worldwide, their abundance and capacity to produce bioactive metabolites depend on their habitat, location, and environment (Sivalingam et al., 2019). Based on ecological differences, bacterial communities differ in their microbial flora (Orland et al., 2019). Hunting et al. (2015) reported that a higher level of biodiversity in an ecosystem necessitates a greater use of resources and vice versa. *Streptomyces* species is one of the multi-species model system soil bacteria that represents diversity and competition among organisms along with their ability to produce secondary metabolites (Alam et al., 2022). Production of novel metabolites as antibiotics by *Streptomyces* in cultivated soil ecosystems with antagonistic potential against some bacteria has been discovered by Khadayat et al. (2020).

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Streptomyces can grow in the humus layer, and different plant species can affect their diversity (Wang et al., 2022). Plants can produce toxic chemicals that attack *Streptomyces*, leading to the production of their own secondary metabolites as a defense mechanism. *Streptomyces* interacts with other microorganisms, producing important enzymes, and changes in the environment can lead to the production of specialized metabolites. Scientists are interested in discovering novel *Streptomyces* in extreme habitats, as new gene clusters can synthesize novel secondary metabolites in novel strains (Liu et al., 2022).

There has been a significant decrease in the number of new *Streptomyces* species and bioactive compounds discovered in recent years. In places like the United Arab Emirates, unexplored terrestrial habitats could be a unique niche for discovering novel species of *Streptomyces* that produce new secondary metabolites with useful bioactivities. Metagenomic analysis has revealed that Actinobacteria are one of the major groups that are found in desert soil (Xie & Pathom-Aree, 2021).

Streptomyces strains from UAE terrestrial habitats were isolated and screened in this study. The capacity of the strains to produce bioactive inhibitory compounds was investigated. Several novel strains were identified and found to be capable of producing antimicrobial and cytotoxic agents.

2. Materials and methods

2.1. Microbial organisms

The test organisms used in this study to assess antimicrobial activity included 5 bacterial strains, *Candida albicans* (ATCC® 66027), and *Saccharomyces cerevisiae* (ATCC® 2601). The bacterial strains were *Staphylococcus aureus* (ATCC® 29213), *Escherichia coli* (ATCC ® 25922), *Bacillus subtilis* (ATCC® 6051). The *E. coli*-ESBL was supplemented by the microbiology lab at the University of Sharjah Hospital (Sharjah, UAE).

2.2. Sample collection and location

Fifteen soil samples were collected from eight cultivated central areas: Al Khawaneej, Al Mizhar, Al Warqa, Al Qusais, Oud Al Mateena, Al Jurain, and Al Dhaid. The coordinates of these locations are shown in Table 1. Using a shovel, samples were taken to a depth of approximately 10 cm after removing 3 cm of the topsoil and about 500 g were collected and then placed in polystyrene bags. Before being processed, the samples were taken to the lab and kept at 4 degrees Celsius. The names of the samples were shortened according to where they came from. The samples were air-dried overnight in a chemical hood. Then, they were heated to 56 °C for 30 min in a water bath to stop the growth of vegetative cells and facilitate the isolation of *Streptomyces*. Sterilized water was used to dilute each sample up to 10^{-5}.

2.3. Isolation and screening of Streptomyces

The soil sample was suspended in sterile water and serially diluted from 10^{-1} to 10^{-5}. On Starch Casein Agar (SCA; Himedia, India) plates, 0.1 mL of 10^{-3}, 10^{-4} 10^{-5} dilutions were spread. The rough, chalky, and firm textures of the *Streptomyces* colonies were determined after incubating the plates at 28 °C for seven to fourteen days. For the purpose of morphological characterization, these isolates were streak-purified on oatmeal and SCA agar plates, and stored as working stocks in the refrigerator.

2.4. Disk and well diffusion method

The disk diffusion method (Kirby Bauer method; Bauer et al., 1959) was used to evaluate the inhibitory activity of the streak-purified isolates, recovered from soil samples, against the test organisms *E. coli*, *S. aureus, C. albicans,* and *S. cerevisiae* using Mueller Hinton agar medium (Himedia, India). Using sterile cork borer, disks of 5 mm in size of the *Streptomyces* colony mass were made from the purified agar stocks (working stock) of the strain. A fresh culture of the test organism with an OD between 0.02 and 0.05 was plated on Mueller Hinton agar plates before placing the disks aseptically. After incubation at 28 °C for 24 to 72 h, the zones of inhibition were measured in millimeters.

The *Streptomyces* isolates' cultures were inoculated into 25 mL of yeast tryptone extract medium (ISP1; Himedia, India) and incubated shaking for 21 days at 28 °C. The cell-free broth's antimicrobial activity against *E. coli*, *S. aureus*, *C. albicans*, and *S. cerevisiae* was evaluated using the well diffusion method. Mycelium and supernatant were separated by centrifugation at 4,000 rpm for ten minutes after spores from pure cultures were inoculated into tryptone yeast extract medium (ISP1) in a volume of 50 mL and incubated at 28 °C for seven to fourteen days. Syringe filters measuring 0.22 µm (Fisher Scientific, USA) were used to filter and collect the cell-free filtrate. Mueller Hinton agar plates were spread with the tested microorganism, and then 5 mm diameter wells were made using the end of a sterile Pasteur pipette. Filtrates of the *Streptomyces* strains were added to the wells in a volume of 50 µL. The inhibition zone's diameter around the well was measured after incubating the plates for 24 h at 28 °C.

Table 1. Isolation of *Streptomyces* strains from different geographical locations across the Sharjah and Dubai regions with GPS coordinates.

Soil sample ID	Location	Emirate	No. of strains	GPS coordinates
CSK	Al Khawaneej	Dubai	3	25°13′38.6″N 55°31′02.4″E
CSM	Al Mizhar	Dubai	2	25°14′29.8″N 55°29′02.5″E
CSW	Al Warqa	Dubai	2	25°10′58.9″N 55°26′37.7″E
CSQ	Al Qusais	Dubai	2	25°15′50.6″N 55°23′55.1″E
CSO	Oud Al Muteena	Dubai	1	25°15′58.1″N 55°27′12.2″E
CSU	University of Sharjah	Sharjah	3	25°17′14.1″N 55°28′49.6″E
CSG	Al Gharayen	Sharjah	1	25°18′09.5″N 55°30′34.2″E
CSD	Al Dhaid	Sharjah	1	25°17′14.1″N 55°53′43.8″E

2.5. Phenotypic and biochemical characterization

Streptomyces strains were characterized physically, biochemically and morphologically according to the International *Streptomyces* Project (ISP) (Shirling & Gottlieb, 1966) and Bergey's Manual of Systematic Bacteriology (Locci, 1989). Melanin synthesis, carbon utilization, gram staining, and the production of hydrogen sulfide were among the physiological and biochemical tests. Morphological growth patterns of the strains were assessed on starch casein agar (SCA). Visually, the color of the aerial mycelium was detected along with substrate mycelium and soluble pigment production. A bacterial smear of each isolate was observed under the light (Olympus compound microscope CX31, Japan) and electron scanning (TESCAN SEM solutions located at the Department of Applied Physics and Astronomy, University of Sharjah) to assess spore surface and chain morphology.

2.6. Molecular characterization

The Promega Wizard® Genomic DNA Purification Kit (Promega, USA) was used to isolate the genomic DNA of CSK1, CSK3, CSW2, CSU1, CSU2, and CSG1 in addition to the negative strain CSM1. Primers 27 F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492 R (5′-TACGGCTACCTTGTTACGACTT-3′) were used to amplify the 1480 bp 16S rRNA. The following were the PCR conditions: initial denaturation for two minutes at 94 °C, followed by 30 cycles of denaturation for one minute at 94 °C, annealing for one minute at 55 °C, extension for one minute at 72 °C, and final extension for three minutes at 72 °C. A 1% Agarose gel electrophoresis using a marker of 1 kb Hyperladder was prepared to determine the size, integrity, and quality of the DNA. The PCR products were sequenced with Applied Biosystems 3500 Series Genetic Analyzers. The 16S rRNA sequence was entered into the NCBI nucleotide blast to determine the identity and similarity of the sequence with other species in the database. Mega 7 software was used for sequence alignment and the generation of phylogenetic trees.

2.7. Preparation of organic extracts

Cultures of *Streptomyces* isolates were inoculated into 25 mL of yeast tryptone extract medium (ISP1) and grown for 21 days. The cell-free broth was filtered using 0.22 μm syringe filters, and the metabolites were extracted using an equal volume of ethyl acetate as the organic solvent. After shaking vigorously for 30 min, the mixture was allowed to stand in a separatory funnel to separate the 2 phases. The sample was placed in an oven at 50 °C to evaporate the organic layer (Elias et al., 2022). The dried extract was then weighed, resuspended in 1 mL of double distilled water, and sterilized using 0.22 μm syringe filters for further analysis.

2.8. Minimum inhibitory concentration (MIC)

The minimum concentration required by the organic extracts to inhibit microbial growth was assessed by the microbroth dilution test was performed using 96 well plates. The resazurin-based turbidometric (TB) assay (Elshikh et al., 2016) was adapted. A 50 μl of Mueller Hinton broth was added to columns 2 to 12. As a sterility control, 100 μL of Mueller Hinton was added to column 1. The organic extracts were serially diluted from well one with a concentration of 30 μg/μL to 0.12 μg/μL (columns 2 to 11). *E. coli*, *S. aureus*, *C. albicans*, *S. cerevisiae*, *B. subtilis*, and *E. coli*-ESBL were the tested organisms, with optical densities standardized to 0.05 at 600 nm to achieve a final volume of 100 μL. The standardized microbial suspension was added to columns 2 through 12, with column 12 serving as a control for microbial growth. After 24 hrs of incubation at 37 °C, 10 μL of 0.1% resazurin was added to the plates, and the absorbance was measured at 630 nm using an ELISA reader (BioTek ELx808, USA). Each isolate was tested against each of the tested organisms in triplicate. GraphPad Prism was used to plot the MIC values (GraphPad Software, La Jolla, California, USA).

2.9. Cytotoxic activity

The human cervical adenocarcinoma cell line (HeLa; ATCC®CCL-2) was cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), and 100 units/mL penicillin/Streptomycin in a 5% humidified CO2 incubator (SheL Lab, USA). Using 0.25% Trypsin-EDTA, cells were subcultured at 70–80% confluency. Three independent replicates of the MTT

(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) assay was performed to measure the extracts' antiproliferative effect. In 100 µL of culture medium, Hela cells were seeded in 96-well plates at a density of $4x10^5$ cells per well and incubated for 24 hrs. Extracts were added to the cells at concentrations of 5, 10, 15, and 20 µg/µL. As a control, untreated HeLa cells were utilized. After incubation for 24 hrs, the medium was replaced with 100 µl of growth medium. MTT reagent (5 mg/mL) was added at a volume of 15 µL and incubated for three hours at 37 degrees Celsius. After that, the colored formazan crystals produced by MTT were dissolved with 100 µL of DMSO, and measured at 570 nm using a microplate reader (BioTek ELx808, USA). GraphPad Prism 7.04 statistical software (GraphPad Software Inc., USA) was used to calculate the IC50.

For DAPI staining, Hela cells were treated with 5 and 20 µg/µL concentrations of each organic extract and incubated for 24 hrs. DAPI staining was performed as follows: fixing the cells with 4% (v/v) paraformaldehyde for 8 min, permeabilization with 0.1% (v/v) Triton-X-100 for ten minutes, and staining with 1 µg/mL DAPI for 5 min, then the cells were washed with phosphate-buffered saline (PBS) after each step, and untreated cells were used as the control. Apoptotic nuclear changes in DAPI-stained cells were observed under the fluorescent microscope (IX53 Olympus, Tokyo, Japan). Apoptotic cells were quantified. The test was performed in three replicates.

2.10. Statistical analysis

The student's t-test was used to conduct the study's statistical analysis. PRISM 7.04 (GraphPad Software) was used to evaluate MIC concentrations.

3. Results

3.1. Isolation and screening of Streptomyces

Fifteen *Streptomyces* strains were isolated from various cultivated terrestrial locations in Sharjah and Dubai, UAE (Table 1). The strains were identified according to the genus description of Shirling and Gottlieb (Shirling & Gottlieb, 1966). The strains were observed as discrete, powdery, velvety, and chalky aerial mycelium, features that are unique to *Streptomyces* (Figure 1). The color of the colonies was white, yellow, brown, and grey, and their sizes ranged from small to medium. Strains were streak-purified on oatmeal agar and SCA media, respectively (Figure 2).

CSK1, CSK3, CSW2, CSU1, CSU2, and CSG1 were isolated from a total of 15 strains. They were able to inhibit *E. coli* and *S. aureus*, using the agar diffusion method, with zones of inhibition (ZOI) ranging between 16 and 26 mm (Table 2, Figure 3). The other

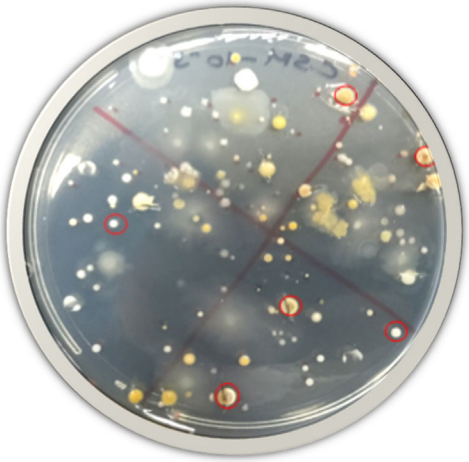


Figure 1. Microbial flora isolated from the soil of Al Khawaneej region (CSK). To isolate *Streptomyces*, the sample was serially diluted with soil (10^{-1} to 10^{-5}), and each dilution was spread on starch casein agar. Dilution 10^{-3} revealed chalky and powdery colonies resembling *Streptomyces* colonies in a variety of colors, including white, beige, yellow, brown, and greyish-black (represented by red circles, B). Each soil sample was processed in the same manner.

strains, such as CSM1, CSM2, CSQ1, CSD1, CSO1, CSQ2, CSK2, CSU3 and CSW2 showed no activity against one or both tested organisms. When tested against *S. cerevisiae* and *C. albicans*, the six strains also displayed greater antifungal activity, with inhibition zones ranging from 17 to 35 mm (Table 2, Figure 4). Isolate CSM1 was used as a negative control throughout this study because it did not have any inhibitory effect against any tested organisms. ISP1 medium was inoculated with isolates that demonstrated disk diffusion inhibitory activity against yeasts and bacteria for 10 to 14 days, and secondary metabolite production was observed and assessed. According to the National Committee for Clinical Laboratory Standards, NCCLS (Coutant et al., 1996), the well diffusion method was used to evaluate the filtered supernatant (filtrate) of each strain using *E. coli*, *S. aureus*, *C. albicans*, and *S. cerevisiae* as the test organisms (Figure 5). The isolates' inhibitory effect was confirmed and correlated in both disk and well diffusion methods.

3.2. Phenotypic and biochemical characterization

The color of the aerial and substrate mycelium, as well as melanin, and a variety of other phenotypic properties were examined (Table 3). One of the features in the classification of *Streptomyces* is the formation of melanin; CSK1 and CSK3 produced light brown melanoid pigments, while CSM1 produced black pigments on ISP7 (Tyrosine agar) medium. All isolates tested positive for catalase and negative for hydrogen sulfide production. The isolates' capacity to utilize various carbon sources was demonstrated,

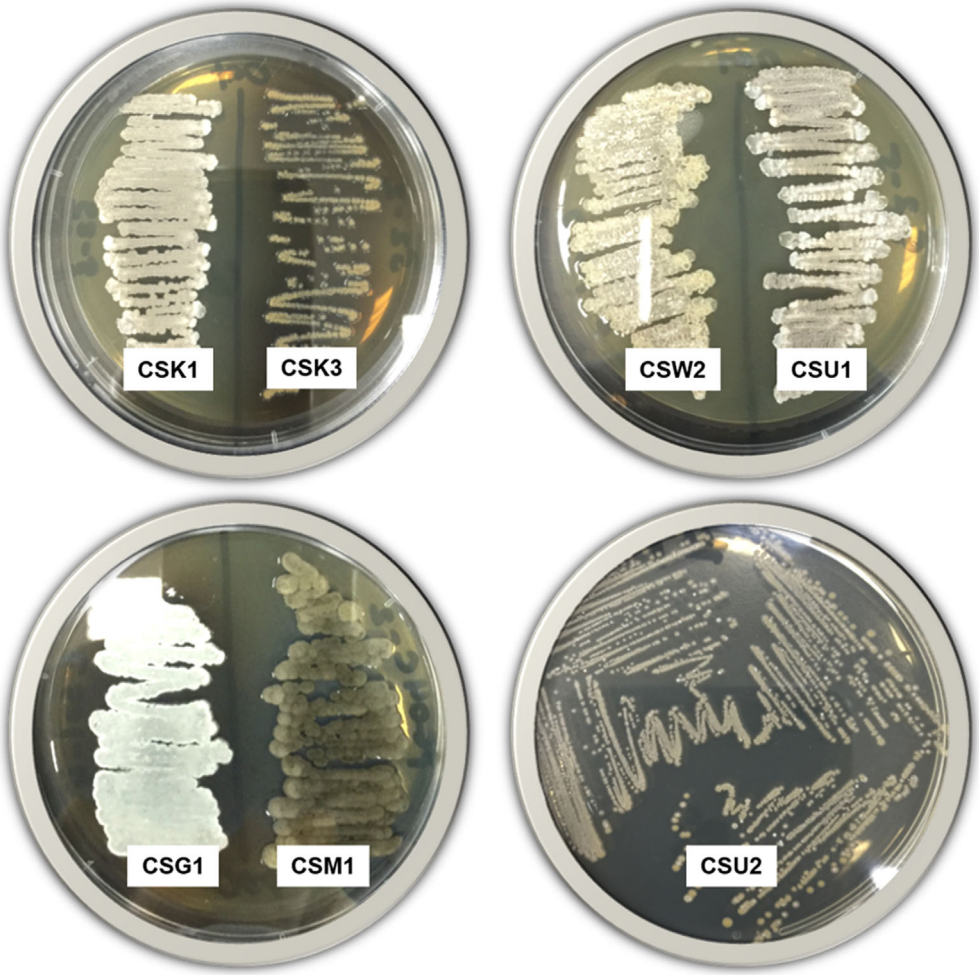


Figure 2. *Streptomyces* streak isolated from soil. For further characterization, isolates with small chalky and velvety colonies were streak purified on oatmeal agar and starch casein medium. According to Shirling and Gottlieb (Shirling & Gottlieb, 1966), the above isolates (CSK1, CSK3, CSW2, CSU1, CSG1, CSM1, and CSU2) observed on starch casein medium exhibit distinct characteristics for *Streptomyces*. These characteristics include an earthy scent brought on by the presence of the volatile metabolite geosmin, which is produced by actinomyces.

Table 2. Testing the antimicrobial activity of *Streptomyces* strains was tested against different microbes using the disk diffusion method.

Isolate	*E. coli*, mm ± SD	*S. aureus*, mm ± SD	*S. cerevisiae*, mm ± SD	*C. albicans*, mm ± SD
CSK1	18 ± 2.08	20 ± 0.58	22 ± 1.73	35 ± 2.31
CSK3	16 ± 0.76	14 ± 0.36	19 ± 0.58	34 ± 0.58
CSW2	16 ± 0.58	18 ± 0.58	20 ± 2.08	35 ± 1.15
CSU1	16 ± 2.89	25 ± 1.15	17 ± 1.53	30 ± 2.08
CSU2	17 ± 0.58	26 ± 1.53	17 ± 0.58	27 ± 0.14
CSG1	20 ± 1.15	17 ± 0.58	20 ± 1.73	34 ± 3.46

The values presented are the mean of three replicates of each experiment, with the standard deviation calculated.

with glucose as a positive control. All isolates, with the exception of CSM1 (the negative control), used inositol, while 60% of the isolates utilized Arabinose, and only 30% of them used xylose or sorbitol.

The taxonomy of *Streptomyces* relies heavily on the characterization of spores, which form when the organism is subjected to stressful conditions and undergoes differentiation in the morphology linked to the production of bioactive metabolites. Examination using scanning electron microscopy to observe the surface features of spores revealed that short chains of rod-shaped morphology were present in all isolates. For CSW2, CSU1, and CSU2, the spore surface texture was found to be spiny, whereas, for CSG1, CSK1, and CSK3, it was found to be smooth (data not shown).

3.3. Gene sequencing and phylogenetic analysis

Genomic DNA was isolated from CSK1, CSK3, CSW2, CSU1, CSU2, CSG1 bacterial cells including the negative control CSM1. To identify the isolates' diversity, taxonomy and evolutionary relatedness, the 16S rRNA fragment of 1480 bp was amplified and sequenced using 27 F and 1492 R universal primers. The16S rRNA gene sequences were BLASTn analyzed

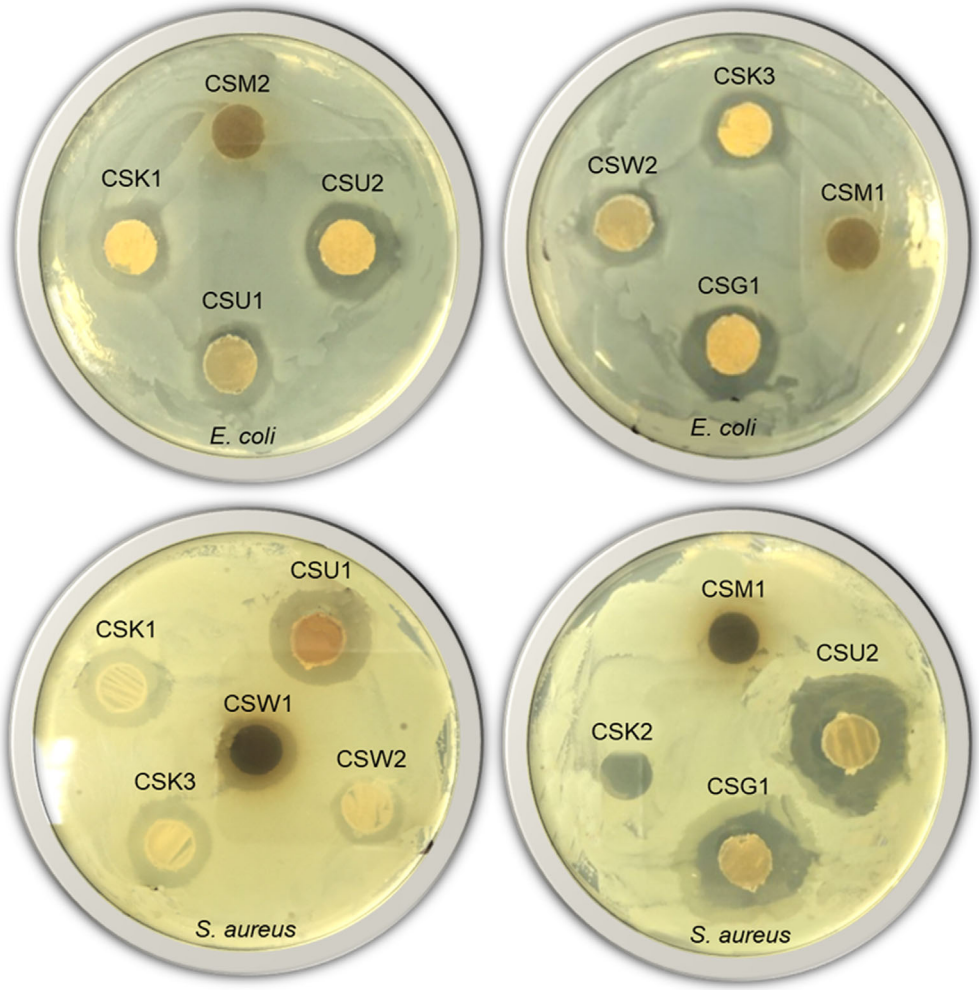


Figure 3. Inhibitory activity of *Streptomyces* isolates against *E. coli* and *S. aureus*. From the streak purified *Streptomyces* isolates on starch casein agar medium, 10 mm-sized disks were cut and placed on Mueller Hinton agar plates that were plated with either *E. coli* or *S. aureus*. Both test bacteria were inhibited by the *Streptomyces* isolates CSK1, CSK3, CSW2, CSU1, CSU2, and CSG1, and growth surrounding the disks was cleared. Isolates CSM1, CSM2 and CSK2 showed no inhibitory effect against both tested bacteria and CSM1 was adopted as the negative control in this study.

to determine the isolated strains against the NCBI GenBank database. The phylogenetic position of the strains was determined by the neighbor-joining method constructed using Mega version X. Closely related *Streptomyces* sequences were retrieved from GenBank and used for the multiple alignments using Clustal W. The 16s rRNA sequence of the isolated bacterial species was aligned with members of the genus *Streptomyces* retrieved from the GenBank database and a phylogenetic tree constructed with well-known *Streptomyces* species (Figure 6), showing that *Streptomyces* strains formed distinct, yet related phyletic lines within the *Streptomyces* species. The gene sequences were registered in Genebank with the following accession numbers: OL845930 (CSK1), OL845931 (CSK3), OL845932 (CSW2), OL845933 (CSU1), OL845934 (CSU2), OL845935 (CSG1) and OL845936 (CSM1). The constructed phylogenetic tree suggests that CSU1, CSG1, and CSW2 are closely related to *S. coelicolor*, CSK1 to *S. variablis* and CSU2 to *S. labedae*, CSK3 to *S. griseorubens* and finally, CSM1 arose from a different node and is closely related to *S. violaceochromo*.

3.4. Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the organic extracts was determined against *E. coli*, *S. aureus*, *C. albicans*, *S. cerevisiae*, *E. coli*-ESBL and *B. subtilis* using the resazurin-based turbidometric (TB) assay (Table 4). All isolates had MIC values between 0.21 and 0.29 µg/mL against *S. aureus*, making them highly inhibitory. However, all isolates had the lowest inhibitory activity for *E. coli*-ESBL, with MIC values between 5.69 and 10.91 µg/mL, except CSK3, which had the lowest inhibition against *S. cerevisiae* at 12.17 µg/mL. The isolates showed variable antagonism against one organism or another, ranging from 0.21 to 12.17 µg/mL.

3.5. Cytotoxic activity

MTT assay and DAPI staining of the organic extracts on HeLa cells were performed to measure apoptosis and antiproliferative effects. Doxorubicin served as a positive control in the MTT assay, while CSM1 served

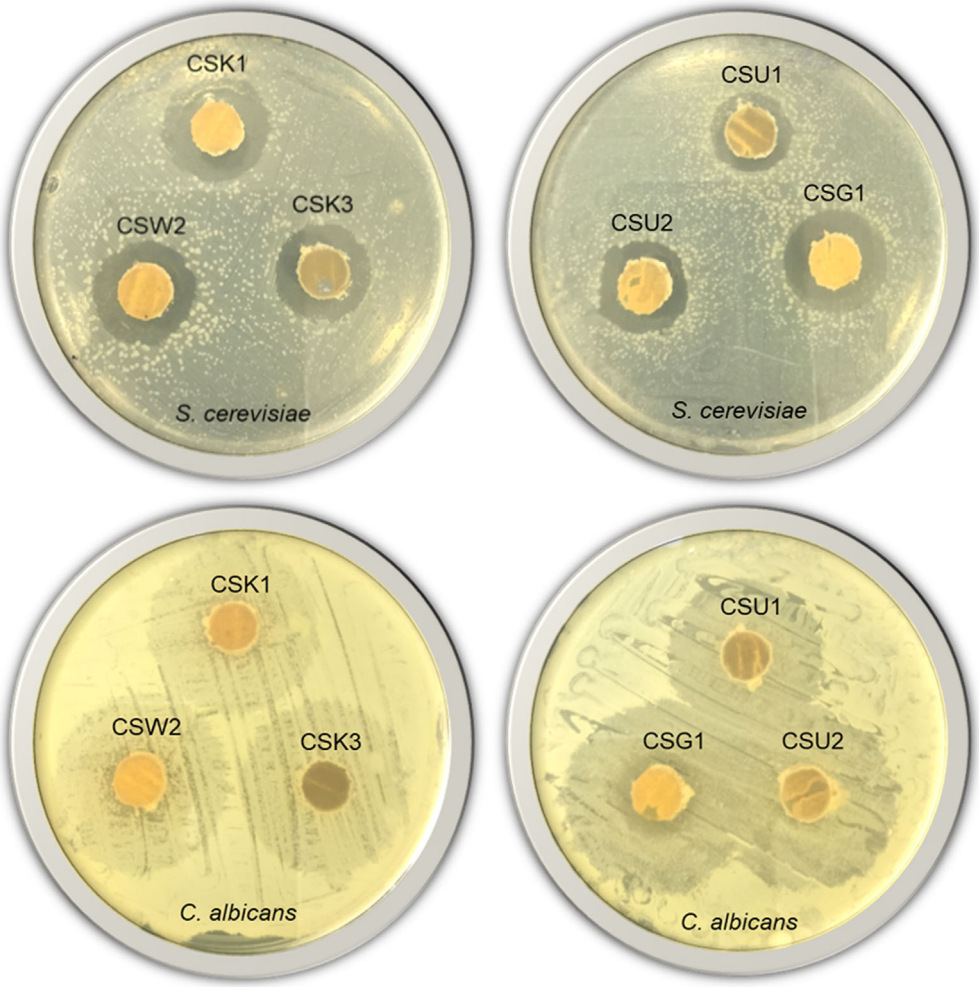


Figure 4. Inhibitory activity of *Streptomyces* isolates against *S. cerevisiae* and *C. albicans*. Ten millimeter disks were cut from streak purified CSK1, CSK3, CSW2, CSU1, CSU2 and CSG1 *Streptomyces* strains grown on SCA. These disks were then placed on Mueller Hinton agar plates that were spread with either *S. cerevisiae* or *C. albicans*. The tested isolates demonstrated inhibitory activity against both organisms.

as a negative control. Most isolates, except for CSK1 and CSU2, experienced a concentration-dependent inhibition of Hela cell growth, with concentrations ranging from 5 to 20 g/μL (Figure 7a). Figure 7(b) shows the IC50 values of isolates exhibiting cytotoxicity toward HeLa cells. IC50 values varied between the examined extracts, with CSK3 showing the lowest IC_{50} value of 3.46 μg/μL while CSU1 had the highest value of 9.74 μg/μL.

By inducing Hela cells with 5 and 20 g/L of *Streptomyces* organic extracts for 24 h, the morphological changes caused by apoptosis were evaluated. Nuclear DAPI staining was used to investigate apoptosis after the cells had been fixed and stained with DAPI. Contrary to the negative extract CSM1 and control (untreated HeLa cells), which displayed normal nuclei, chromatin condensation and DNA fragmentation were clearly observed in CSK3, CSW2, CSU1, and CSG1 specifically at 20 μg/μL (Figure 8a). Isolates of CSK1 and CSU2 did not show signs of apoptosis. In order to determine the percentage of nuclear fragmentation, Hela cells were counted. As seen in Figure 8(b), a higher dosage of the extracts causes more significant changes to the nuclear structure.

4. Discussion

The search for novel bioactive compounds is becoming crucial as microbial resistance to antibiotics has been continuously increasing to fight multi-drug resistant pathogens. The *Streptomyces* genus is an important source of many bioactive compounds (Quinn et al., 2020). However, it is becoming hard to find new secondary metabolites from *Streptomyces*. *Streptomyces* isolated from terrestrial and aquatic habitats are an important supply of bioactive compounds (Al-Shaibani et al., 2021). Therefore, the focus on finding a new group of rare and novel *Streptomyces* strains from unusual ecosystems is explored in this research as a potential source to identify new therapeutic compounds.

A total of 15 *Streptomyces* isolates were found in eight soil samples taken from various locations in

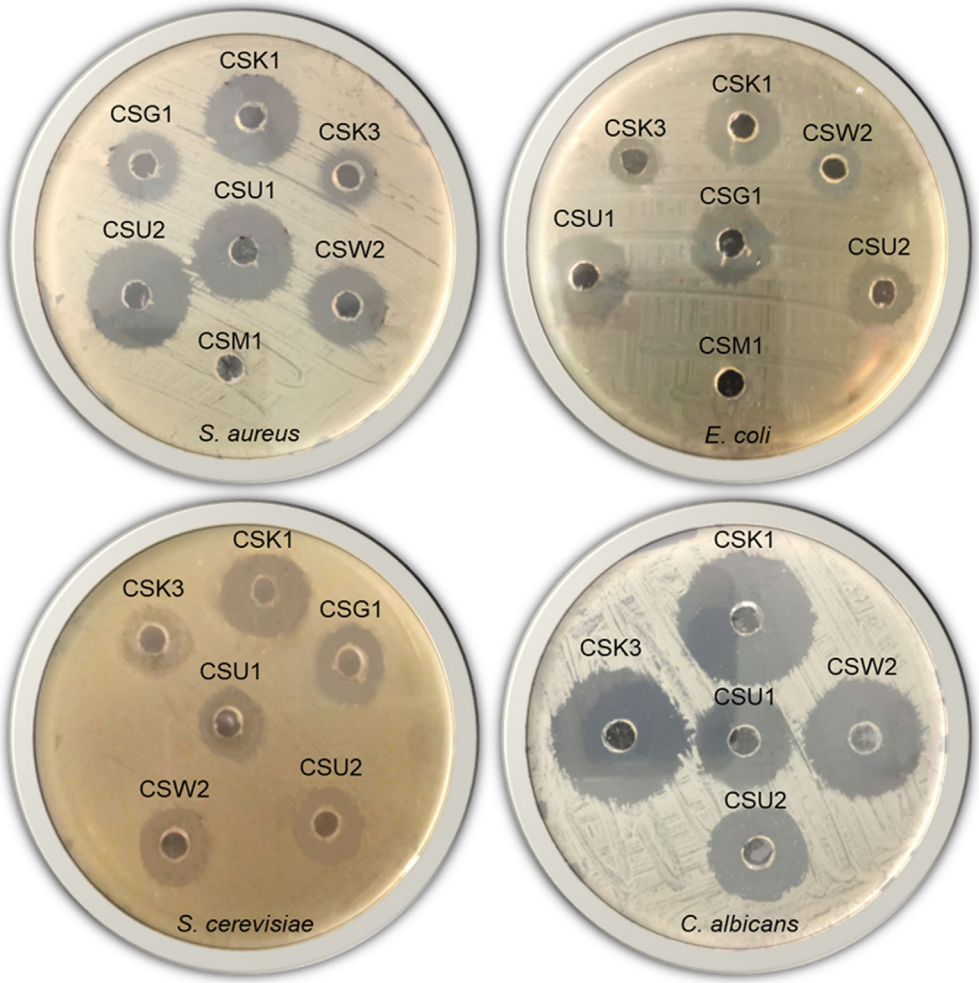


Figure 5. Inhibitory effect of *Streptomyces* isolates by well diffusion method. The fermented culture broth filtrates was used to isolate *Streptomyces* strains. Using Mueller hinton agar medium, the filtrates were tested against *E. coli*, *S. aureus*, *S. cerevisiae*, and *C. albicans* using the well diffusion method. Except for CSM1, which is the negative isolate, all of the filtrates (CSK1, CSK3, CSW2, CSU1, CSU2, and CSG1) demonstrated inhibitory activity against the organisms that were tested.

Table 3. Morphological and biochemical characteristics of *Streptomyces* strains.

Characterization*	*Streptomyces* Isolates						
Isolates	CSK1	CSK3	CSW2	CSU1	CSU2	CSG1	CSM1
Color of aerial mycelium	White	White	White	White	White	White	Yellow
Color of substrate mycelium	Yellow	Grey	Yellow	Grey	Grey	Yellow	Yellow
Soluble Pigment	–	Brown	–	–	Brown	Brown	–
Melanoid Pigment	Light brown	Light brown	–	–	–	–	Black
Carbon source utilization							
Inositol	+	+	+	–	+	+	+
L-Arabinose	+	+	+	–	–	–	+
xylose	–	–	+	–	–	–	+
Sorbitol	–	–	+	–	–	–	+

The results are presented as either positive (+) or negative (−). All tests were performed in triplicates.
*All *Streptomyces* isolates were catalase positive and hydrogen sulfide negative.

the eastern United Arab Emirates. The UAE has a scorching desert climate, with temperatures frequently hovering between 40 and 50 °C for most of the year, except for a short two-month winter period where temperatures drop below 20 °C. According to Airey et al. (2021), rainfall in the United Arab Emirates is uncommon and short-lived. During the winter season, rain occurs in the form of downpours, with an annual precipitation of less than 100 millimeters. Sandstorms, which can last several days and facilitate horizontal gene transfer between bacterial communities during the summer, are another important factor to consider (Behzad et al., 2018). Dust and sandstorms have been regarded as vehicles of transport for microorganisms (Opp et al., 2021) and many bacteria and fungi were identified in these events (Nourmoradi et al., 2015). Most of the microorganisms transported are highly resistant and able to live in harsh environmental conditions with low nutrition and water levels, and extreme temperatures (Behzad et al., 2018). Desert microbes have an extensive reservoir of genes associated with osmoregulation

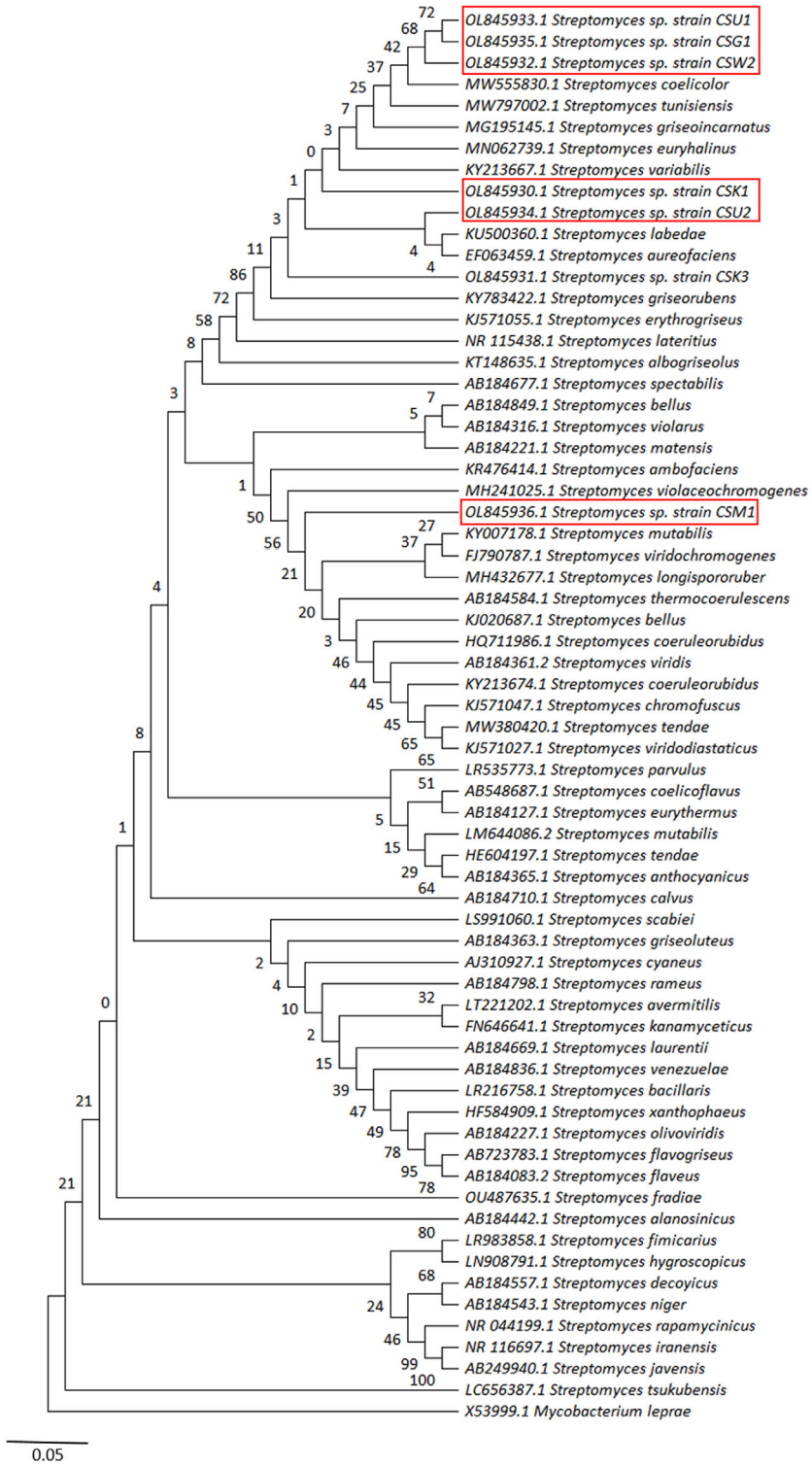


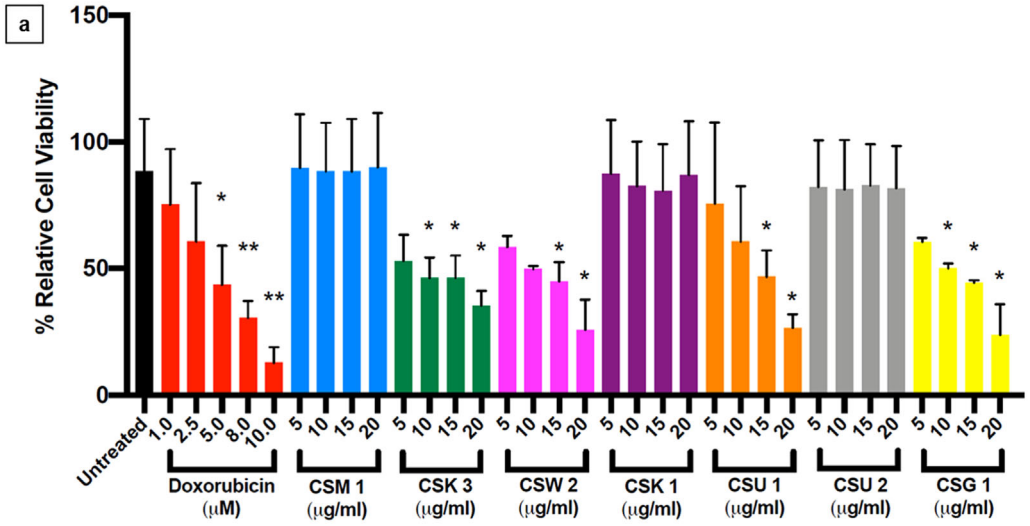
Figure 6. Phylogenetic tree illustrating the evolutionary relatedness of seven *Streptomyces* strains (CSK1, CSK3, CSW2, CSU1, CSU2, CSG1, and CSM1) isolated in this study, based on their 16S rRNA gene sequences. The tree was constructed using the maximum likelihood method and the bootstrap values were calculated based on 500 replicates, represented as percentage values on the branch nodes with *Mycobacterium leprae* used as an outlier organism. Multiple alignments were performed with Clustal W software. The scale bar indicates a 0.050 substitution position. The names of the strains and their corresponding GenBank accession numbers are provided on the tree and denoted by red triangles.

and dormancy, which play a significant role in their survival under such harsh conditions (Alsharif et al., 2020).

In our study, eleven out of the 15 isolates had activity against one or both of the tested bacteria, which is more than 70%, with six of the isolates (40%) exhibiting an inhibitory effect against all tested microorganisms. The secondary metabolites produced by the *Streptomyces* isolates account for their inhibitory effect against the tested organisms. The larger the zone of inhibition, the stronger is the response generated by the isolate to the tested

Table 4. Minimum inhibitory concentration (MIC) of *Streptomyces* isolates was assessed using the resazurin-based turbidometric (TB) assay with 3 independent replicates, and the values were quantified by the GraphPad Prism 7 software using the Gompertz model (Lambert & Pearson, 2000).

	Concentration μg/mL ± SD					
Isolate	*E. coli*	*S. aureus*	*C. albicans*	*S. cerevisiae*	*B. subtilis*	*E. coli*-ESBL
CSK1	4.03 ± 0.01	0.21 ± 0.01	1.97 ± 0.01	7.32 ± 0.01	2.54 ± 0.01	9.07 ± 0.02
CSK3	1.91 ± 0.04	0.23 ± 0.02	4.61 ± 0.01	12.17 ± 0.01	6.53 ± 0.01	6.16 ± 0.02
CSW2	4.72 ± 0.04	0.29 ± 0.02	4.13 ± 0.01	9.33 ± 0.01	3.58 ± 0.003	8.60 ± 0.03
CSU1	1.97 ± 0.02	0.24 ± 0.02	3.98 ± 0.01	3.36 ± 0.01	7.36 ± 0.01	10.64 ± 0.03
CSU2	1.97 ± 0.02	0.21 ± 0.01	4.25 ± 0.01	7.10 ± 0.02	10.91 ± 0.02	5.69 ± 0.01
CSG1	1.95 ± 0.01	0.23 ± 0.01	4.67 ± 0.01	9.35 ± 0.03	5.69 ± 0.02	10.91 ± 0.01



b

Streptomyces isolates	IC_{50} (μg/μl) (mean & error ± SD)
CSK3	3.46 ± 0.19
CSW2	5.73 ± 0.09
CSU1	9.74 ± 0.08
CSG1	6.34 ± 0.07

Figure 7. Cytotoxicity of *Streptomyces* extracts on HeLa cells. The cytotoxicity of treating HeLa cells with *Streptomyces* organic extracts at various concentrations (ranging from 5 to 20 μg/μl) was evaluated. The MTT assay was used to measure the cancer cells' cytotoxicity at an absorbance of 570 nm (a). Strain CSM1 served as a negative control, and doxorubicin as a positive control. The results presented are means of standard deviation from three independent experiments. T-test was used to compare two samples assuming equal variance ($^{*}p < 0.05$ and $^{**}p < 0.005$) and the data were normalized to the mean. Dose response (IC_{50}) values were determined for strains that showed toxicity by MTT assay (CSK3, CSW2, CSU1 and CSG1) using Graphpad prism software (b). IC50 values based on the concentration of the crude extract concentration required to reduce the HeLa cancer cell line by 50% and represent mean ± SD ($n = 3$).

organism. The differences in the zones of inhibition generated depend on the secondary metabolites that the isolates produce, where each isolate produces different types of secondary metabolites (Thirumurugan et al., 2018) that consist of other chemical compounds. Because they do not influence the organism's growth and are not involved in cell division, they are referred to as "Secondary." The majority of secondary metabolites are produced when *Streptomyces* moves from a vegetative state to sporulation and aerial multinucleoid mycelium during the life cycle (Manteca et al., 2019). Stressful environmental conditions like space, competition with other organisms, temperature, pH, salt changes, or physiological signaling molecules can cause this morphological differentiation (Boruta, 2021). The organism's secondary metabolism is activated during *Streptomyces'* stationary growth phase.

An important phenotypic feature for identifying the *Streptomyces* genus is the distinct colony morphology with chalky, powdery, and velvety aerial mycelium that was evidently observed in the isolated strains. The isolates showed varied cultural and morphological characteristics observed in pigment production and the aerial and substrate mycelium color. All of the isolates were negative for the production of hydrogen sulfide and positive for catalase, which can break down oxygen metabolism-related toxins. Nevertheless, their distinct patterns of carbon utilization may suggest that they are distinct strains.

Sequence analysis of the 16s rRNA sequences further confirms the identification of *Streptomyces*

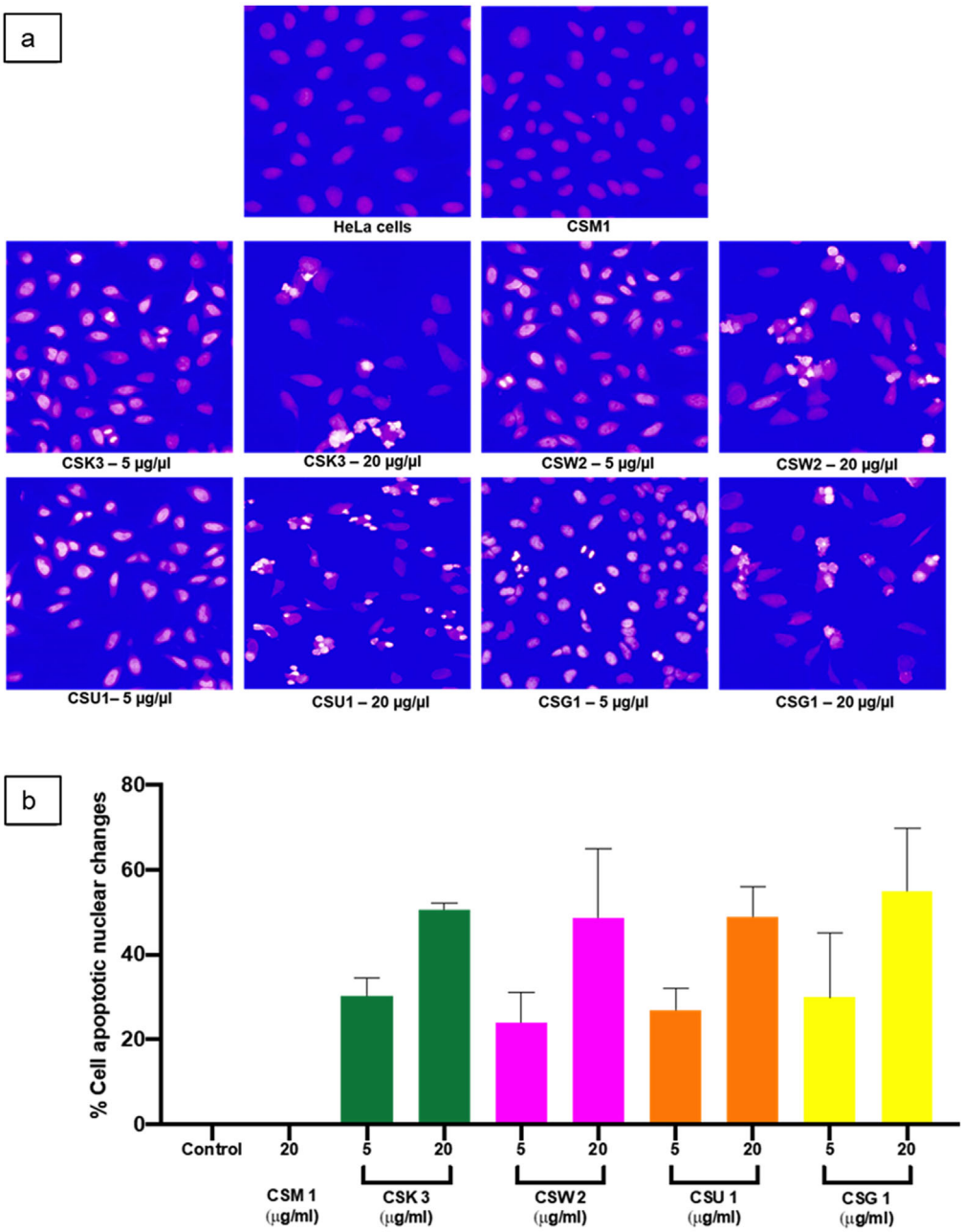


Figure 8. *Streptomyces* organic extracts induce apoptosis of HeLa cells. To assess apoptosis, *Streptomyces* ethyl acetate organic extracts, including the CSM1 negative control strain, were cultured in HeLa cells in the absence of extract (control) and in the presence of 5 and 20 µg/µl, respectively. The cells were fixed and stained with DAPI after 24 h of incubation. Under a fluorescent microscope, CSK3, CSW2, CSU1, and CSG1 strains showed evidence of nuclear fragmentation. These *Streptomyces extracts* induced morphological changes in Hela cells, which were observed (a) and quantified (b). The mean standard deviation of three experiments is used to represent apoptotic nuclear changes.

species. The molecular characterization of these isolates revealed that the sequence similarities with *Streptomyces* species in the Genbank database were between 97–99%. These results may indicate that these isolates belong to different species and could be considered novel strains. Most of the identified isolates are closely related to one of these *Streptomyces* species that are capable of producing antimicrobial products. Three of the isolates (CSU1, CSG1 and CSW2) were found to be closely related to *S. coelicolor*. A literature search on blasted organisms revealed that *S. coelicolor* is a source of more than 20 secondary metabolites, such as Actinorhodin and Germicidin (Ferdous et al., 2020). Isolate CSK1 and CSU2 were found to be closely related to *S. variabilis* and *S. labedae*, respectively. *S. variabilis* was reported to produce an immunosuppressive agent (Abd-Alla et al., 2016) and 1-hydroxy-1-norresistomycin (Ramalingam & Rajaram, 2016); while *S. Labedae* is a bacterium of no known antimicrobial activity. Finally, CSM1 is closely related to *S. violaceochromogenes* reported to produce arugomycin (an anthracycline antibiotic; Ashu et al., 2019).

The resazurin-based turbidometric (TB) assay was also performed to assess the isolates' antimicrobial properties by determining the minimum inhibitory

concentration values against various pathogenic organisms. The findings demonstrated that all isolates are able to inhibit yeast and bacterial growth, with low activity against *S. cerevisiae* and multidrug-resistant isolate *E. coli*-ESBL, moderate activity against *E. coli* and *C. albicans*, and high activity against *S. aureus*. Numerous studies support these findings (Cwala et al., 2011; Nurkanto et al., 2012), demonstrating that streptomycetes have good activity against Gram-positive bacteria but little or no activity against Gram-negative bacteria. This could be because of the biochemical structure of Gram-negative bacteria's outer membrane, which contains LPS (lipopolysaccharide), which renders the cell wall inaccessible to lipophilic compounds and shields the bacteria from some antibiotics.

Cancer accounts for the greatest number of deaths worldwide, with an estimated 10 million in 2020 (Ferlay et al., 2020). *Streptomyces'* ability to produce metabolites that serve as anti-cancer agent has been demonstrated in numerous studies (Ashu et al., 2019). The extracts' cytotoxic activity was first observed by the morphological changes of the HeLa cells. The majority of the cells had an angular and spindle shape (epithelial) and were observed to be adherent. Nevertheless, after treatment with increasing extract concentrations, many cells lost these characteristics. The extracts from CSK3, CSW2, CSU1 and CSG1 showed a decrease in HeLa cell viability with increasing concentration, ranging from 5 to 20 μg/μl. Apoptotic bodies and cell rounding and detachment were observed, indicating that the extracts had a toxic effect on the cells. Cell shrinkage and pyknosis, both of which are the outcomes of chromatin condensation, are thought to be responsible for the earliest changes observed during apoptosis. Following the formation of apoptotic bodies, which separate cell fragments, the nucleus of an apoptotic cell contains dark purple chromatin fragments (Elmore, 2007). HeLa cells treated with CSK3, CSW2, CSU1, and CSG1 extracts showed clear evidence of DNA fragmentation, nuclear condensation, and growth inhibition.

5. Conclusions

This study shows that the UAE holds a promising terrestrial habitat, demonstrating the distribution and diversity of novel *Streptomyces spp*. We have presented the isolation of novel *Streptomyces* species and their potential to produce bioactive secondary metabolites with antimicrobial and cytotoxic properties. Further characterization and identification of the produced secondary metabolites are required as they could be the source for discovering novel and bioactive drugs.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Ethical statement

No experiments were performed on animals and no data were collected from patients in this research.

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